



Investigation of vascular endothelial growth factor (VEGF) polymorphism in patients with idiopathic heavy menstrual bleeding

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Abstract

Purpose To investigate whether there is a relationship between the *VEGF* polymorphisms and idiopathic heavy menstrual bleeding (HMB-E) Query.

Methods Sixty-five patients diagnosed with HMB-E according to the FIGO classification system and 65 female healthy volunteers were included in the study. The polymorphic regions rs699947 (–2578C > A), rs1570360 (–1154G > A), rs2010963 (+405G > C), rs3025039 (+936C > T), rs25648 (c534C > T) in the *VEGF* were detected using Next Generation DNA Sequencing method.

Results The –2578C > A polymorphism CC genotype, CA + AA genotypes, and C allele, as well as the –1154G > A polymorphism AA genotype, and A allele were associated with increased risk of HMB-E ($p < 0.05$ for all). However, no statistically significant difference was found between the patient group and the control group in terms of genotype and allele distributions in the 405G > C, +936C > T, c534C > T polymorphic regions ($p > 0.05$ for all). While the –2578/–1154/+405/c534 AGGC haplotype decreased the risk of HMB-E, the CAGC haplotype was found to increase the risk of HMB-E.

Conclusion *VEGF* –2578C > A and –1154G > A polymorphisms were significantly associated with the risk of HMB-E in the Turkish population.

Keywords Excessive menstrual bleeding · Polymorphism · Vascular endothelial growth factor · VEGF gene

Introduction

Heavy menstrual bleeding (HMB) is an important health problem defined as menstruation with excessive flow and duration at regular cycle intervals. Clinically, it is defined as a blood loss of more than 80 ml per cycle. It is a common gynecological problem among women of reproductive age and accounts for more than 20% of outpatient visits to gynecologists [1]. Although HMB is often associated with uterine pathology (e.g., fibroid, polyp, adenomyosis, carcinoma), abnormal blood clotting or disruption of hormonal regulation, approximately 50% of HMB cases occur in the absence of recognized uterine pathology [2, 3], suggesting a

defect in the cellular processes and regulatory mechanisms of menstruation. Such idiopathic HMB of endometrial origin (HMB-E) has been estimated to account for 9–14% of gynecological outpatient visits [4, 5].

HMB can lead to iron deficiency anemia. Current treatment options often compromise fertility and may require hysterectomy in patients who do not respond to the treatments [6]. Another problem is increased maternal morbidity and mortality for pregnant women who have HMB-associated anemia. Heavy bleeding can affect daily activities. The need for long-term medication, the side effects of these therapies, and the intolerance can cause psychological and social discomfort. Also, for numerous female individuals, problems related to employment have led to the designation of HMB as a public health problem [4].

Disturbance of endometrial angiogenesis is one of several proposed underlying mechanisms that have been suggested to play a role in HMB [4, 5]. Endometrial angiogenesis occurs during all phases of the menstrual cycle and in different layers of the endometrium. Important events during endometrial development angiogenesis include increased

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vascular permeability, and successful remodeling of spiral arteries. [7]. Vascular endothelial growth factor (VEGF) is the main regulatory factor that controls angiogenesis [8].

In the formation of functional and stable endometrial microvessels, covering the luminal surface of endothelial tubes with mesenchymal pericytes is an important step. The pericytes interact tightly and adhere to the endothelial cells with the pores in the basal layer [9]. Pericytes may induce angiogenesis with Vascular Endothelial Growth Factor (VEGF) production which stimulates endothelial cell proliferation and tube formation. Previous studies have shown that the number of capillaries expressing VEGF-A and its receptors (VEGFR-1 and VEGFR-2) in the biopsy specimens of women with HMB were significantly higher compared to healthy controls [1], suggesting that the mesenchymal pericytes enabling angiogenesis may contribute to vascular fragility. It is known that glandular luminal surfaces of patients with HMB are wider and this finding is highly correlated with overexpression of VEGF-A and VEGFR-1 [10]. Inappropriate coverage of the laminal surface of endothelial tubes leads to extensive endothelial cell gaps and abnormal vascular remodeling, causing excessive blood loss [10, 11].

Polymorphisms in regulatory regions of genes can alter gene expression through their effects on transcription or RNA stability. It has been shown that there is a relationship between these functionally important polymorphisms and VEGF protein production [12].

VEGF polymorphism has been associated with many diseases in which angiogenesis is critical, such as cancers, cardiovascular diseases, essential hypertension, diabetes mellitus, and psoriasis, and endometriosis [13]. However, in the literature, there is no study investigating the *VEGF* polymorphism in patients with idiopathic HMB-E. The evidence of the presence of *VEGF* polymorphisms in patients with HMB-E may contribute to our understanding of the role of VEGF in endometrial angiogenesis, and thus may provide new opportunities for future research and treatment options. Thus, patients and doctors will avoid the risks of unnecessary surgeries. We, therefore, planned to investigate the relationship between *VEGF* polymorphism and idiopathic HMB-E.

Materials and methods

Our study is a cross sectional study conducted between February 2019 and March 2020. The ethics approval was obtained from the Ankara Yıldırım Beyazıt University Yenimahalle Training and Research Hospital Clinical Research Ethics Committee (29.01.2019—no. 2019/01/01), and the study was conducted in accordance with the Declaration of Helsinki, including current revisions and the Good Clinical Practice guidelines.

The power analysis of the study showed that 128 patients were needed to achieve 80% power when α error was set at 0.05, β error at 0.05, and effect size at 0.50.

A total of 65 patients between the ages of 18–45 years, who were admitted with the complaint of abnormal uterine bleeding and diagnosed with idiopathic HMB according to the FIGO Classification System [14] and 65 voluntary women with regular menstruation were included in the study. All participants were informed about the study and the consent forms were signed.

The participants were selected according to the defined inclusion criteria: being 18–45-years old, having a regular menstrual cycle between 24 and 38 days, not using hormonal or intrauterine contraception for at least three months prior to the study, not having any abnormal ultrasonographic or hysteroscopic findings, not being pregnant and not lactating.

Women were excluded from the study if they had a uterine pathology (such as a polyp, adenomyosis, leiomyoma, malignancy, hyperplasia), a history of significant medical problems (such as coagulopathies, hypothyroidism, hyperprolactinemia, Polycystic ovary syndrome, endometriosis), if they were smokers, or were taking any medicines that increase the tendency to bleeding.

For patient selection according to these criteria, all participants were evaluated based on a complete medical, gynecological, and obstetric history and gynecological examination with ultrasound assessment. Additionally, hysteroscopy or dilatation and curettage were performed in the presence of suspicion of endometrial pathology.

Menstrual blood loss was evaluated with Pictorial Blood loss Assessment Chart (PBAC) which has a positive predictive value of 85.9% in diagnosing menorrhagia and a good correlation to the alkaline hematin method [15, 16]. Self-assessed PBAC consists of diagrams representing different soiled pads or tampons each day and pads are scored according to the degree of staining and the size of the clots on them. Women with a PBAC score > 100 were included in the HMB group and healthy, ovulating women with a PBAC score < 100 were considered in the control group.

Single nucleotide polymorphism (SNP) selection

Five highly plausible candidate SNPs in the *VEGF* were selected, including rs699947 (– 2578C > A, in the promoter region), rs1570360 (– 1154G > A, in the promoter region) rs2010963 (+ 405G > C, at 5'UTR), rs3025039 (+ 936C > T, at 3'UTR), rs25648 (c534C > T, at exon1) and checked in Ensemble, dbSNP database.

Peripheral venous blood samples were collected from all subjects and transferred to EDTA tubes. Samples were stored frozen at – 20 °C until they were analyzed.

Laboratory studies were carried out in the INTERGEN Genetic and Rare Diseases Diagnosis Research and Application Center.

DNA extraction and genotyping

DNA extraction was made from 200 µl peripheral blood using the spin column method with the HibriGen Blood DNA isolation kit (İstanbul, Turkey). The concentrations of the isolates were measured with Nanodrop 1000 (Thermo Inc.) microvolume spectrophotometer and the study continued with samples containing DNA at a concentration of 10 ng/µl or higher.

Primers were designed for PCR amplification of all coding regions and exon–intron junction regions in the *VEGFA* and 4 polymorphisms (rs699947, rs1570360, rs2010963, rs3025039). Primers, amplicon size, and usage purposes are shown in Table 1.

Amplification of the regions was achieved by using the isolated DNA and the designed primers in the PCR reaction. Amplification efficiency was evaluated by visualizing the reaction results with 2% agarose gel electrophoresis. PCR pools were created by combining the PCRs of each sample and then purified by NucleoFast® 96 PCR kit (MACH-EREY–NAGEL GmbH). The purified samples were quantitated by a spectrophotometer (Nanodrop N1000, Thermo Inc.). The concentration of DNA was determined and diluted to 5 ng/µl.

Detection of polymorphisms was performed by NGS (Next Generation DNA Sequencing) method using Miseq device (Illumina Inc. San Diego, CA, USA). Standardized

samples were prepared for next-generation sequencing using the Nextera XT sample preparation kit (Illumina Inc.). DNA Library preparations were made according to the established instructions. A sample worksheet file was created to introduce the study to the device. All of the prepared samples were loaded into the sample loading compartment in the Miseq Reagent Kit v2 2 × 150 (MS-102-2002, Illumina Inc.) cartridge. After all the preparations and controls were made, the Miseq device was started. Approximately 24 h later, study results were taken and analyzed. Alignment of the obtained readings was done with Miseq Reporter (Illumina Inc.) software on the human genome hg19 version. Analysis of the aligned bam files was performed using IGV 2.3 (Broad Institute) software.

Statistical analyses

Continuous variables were first inspected for normality of statistical distribution graphically and by Shapiro–Wilk test. Data were reported as mean ± standard deviation (SD), median with interquartile ranges (IQRs), or numbers and percentages, as appropriate. The data were analyzed using the Student's *t*-test, Mann–Whitney *U* test, or Chi-squared test to determine the significance of the differences between the groups. The Chi-squared test was used to assess the variation in each SNP frequency from Hardy–Weinberg equilibrium (HWE). Logistic regression analysis models were used to calculate the odds ratio (OR) and 95% confidence interval (CI), thereby assessing the effect of genotypes on HMB risk. These analyzes were calculated with IBM SPSS 25 and the Epitools package in the R 3.6.1 software. The

Table 1 Primers, amplicon size and usage purposes

Primer	Orientation	Sequencing	Genotyping	Amplicon size (bp)	Sequence (5'–3')
VEGFA_SNP2_F	Forward	Exon 1	SNP 2	1500	CGGATGGGTAATTTTCAGGCTGTG
VEGFA_1R	Revers		SNP 3 SNP 5		CCTCCCCCTGCACCTAAGACGAC
VEGFA_2F	Forward	Exon 2	–	468	GGGAAGTGTGGGGAAGGTACAGG
VEGFA_2R	Revers				CTTCCCCTTCCTCCCCACAGTTC
VEGFA_3F	Forward	Exon 3	–	433	GAAGCTCCAAAGAGTGGCATTACAG
VEGFA_3R	Revers				ACCATACCTCCCCTGTGCAACTC
VEGFA_4-5F	Forward	Exon 4	–	975	AGGAGTCAGGGTGAAGCCTAGAGAG
VEGFA_4-5R	Revers	Exon 5			CACTAGCCCTAGAGCCATCATCCTG
VEGFA_6F	Forward	Exon 6	–	398	AGAGACCACCTGCCTCCTGACAC
VEGFA_6R	Revers				TCTACCCGTTGGTGCCAATTAGTG
VEGFA_7F	Forward	Exon 7	–	429	CTGTAGCGCTCGGATCCTTCCAG
VEGFA_7R	Revers				ACCAGGCAGGCAGCTAGAACTGAG
VEGFA_8F	Forward	Exon 8	SNP 4	463	CCTTCCTGTCCTCTCTGCTCTTATGG
VEGFA_8R	Revers				ATCTTCCGGGCTCGGTGATTTAG
VEGFA_SNP1_F	Forward	–	SNP 1	250	CTGGAGCGTTTGGTTAAATTGAGG
VEGFA_SNP1_R	Revers				CCCCATCCCATTCTTGCATATAGG

haplotype of VEGF polymorphisms was analyzed by using SHEsis. The linkage disequilibrium of genotype and allele pairs was calculated via the Haploview version 4.2 (Broad Institute, Cambridge, MA, USA). Statistical significance was set at $P \leq 0.05$.

Results

Baseline characteristics

The baseline characteristics of the subjects are summarized in Table 2. There were no differences between the analyzed groups in terms of age, Body Mass Index (BMI), marital status, gravidity, parity, age at menarche, length of the menstrual cycle. As expected, the patients in the HMB group had significant increases in menses duration and PBAC score compared with the control group.

Genotype and allele distribution of VEGF polymorphisms

The genotype frequency distribution of the selected sample was found to be compatible in Hardy–Weinberg equilibrium ($p > 0.05$), suggesting that the selected sample represents the population.

As shown in Table 3, the risk of HMB was 3.05-fold greater in individuals with the *VEGF* – 2578A > C polymorphism CC genotype, 2.35-fold greater in individuals with the AC + CC genotype than those with the AA genotype, and 1.75-fold greater in individuals with the C allele than those with the A allele (CC vs AA, OR 3.05, 95% CI

1.11–8.84, $p = 0.025$; AC + CC vs AA, OR 2.35, 95% CI 0.97–6.0, $p = 0.051$; C vs A, OR 1.75, 95% CI 1.07–2.88, $p = 0.024$). Compared with the – 1154G > A polymorphism GG genotype, AA genotype was associated with 3.49-fold greater risk of HMB, and A allele was associated with 1.74-fold greater risk of HMB than G allele (AA vs GG, OR 3.49, 95% CI 0.89–18.10, $p = 0.06$; A vs G, OR 1.74, 95% CI 1.01–3.01, $p = 0.04$). However, there was no statistically significant difference between the groups in terms of genotype and allele frequency distributions of rs2010963, rs3025039, and rs25648 ($p > 0.05$ for all).

Haplotype analysis for the association between VEGF gene polymorphisms and HMB risk

Linkage disequilibrium analysis including four SNPs (– 2578C > A, – 1154G > A, + 405G > C and 534C > T) in the *VEGF* revealed one SNP block (Fig. 1).

We detected four main haplotypes, CACC, AGGC, CAGC, AAGT, with frequencies greater than 0.03. These haplotypes were analyzed with the online software SHEsis and compared between the HMB and the control group (Table 4). The AGGC haplotype was found to be statistically significantly lower in the HMB group (23.8%) than in the control group (38.5%) (OR 0.505, 95% CI 0.295–1.517, $p = 0.012$). The CAGC haplotype was statistically significantly higher in the HMB group (25.4%) than the control group (9.2%) (OR 3.387, 95% CI 0.481–2.116, $p = 0.0005$). As a result, it was concluded that the AGGC haplotype decreased the risk of HMB and CAGC haplotype increased the risk of HMB.

Table 2 Baseline characteristics of study subjects

Variables	HMB group ($n=65$)	Control group ($n=65$)	$t/z \chi^2$	P
Age (year)	30.58 ± 7.06	29.08 ± 6.56	$t = 1.261$	0.210
Body Mass Index (kg/m ²)	22.35 ± 2.57	22.77 ± 2.65	$t = 0.923$	0.358
Marital status				
Married	43 (% 66.2)	40 (% 61.5)	$\chi^2 = 0.300$	0.584
Unmarried	22 (% 33.8)	25 (% 38.5)		
Gravidity	1 (2)	1 (1)	$Z = -1.361$	0.180
Parity	1 (2)	1 (2)	$Z = -1.185$	0.290
Age at menarche (year)	13 (2)	13 (1)	$Z = -1.311$	0.190
Menses duration (days)	7 (2.5)	5 (2)	$Z = 5.339$	< 0.001*
Length of menstrual cycle (day)	28 (3)	28 (2)	$Z = 0.320$	0.749
PBAC score	258 (246)	50 (44)	$Z = 9.937$	< 0.001

Data are mean ± SD, median (IQR) or numbers (n) and percentages (%)

t = Student's t test, Z = Mann–Whitney U test, χ^2 = Chi-squared

PBAC Pictorial Blood Loss Assessment Chart

* p values ≤ 0.05 were considered statistically significant

Table 3 Logistic regression analysis of associations between VEGF polymorphisms and risk of HMB

	AMK-E group, <i>n</i> = 65 (%)	Control group, <i>n</i> = 65 (%)	OR (95% CI)	<i>p</i>
rs699947 (− 2578A > C)				
AA	9 (13.8)	18 (27.7)	Reference	
AC	31 (47.7)	31 (47.7)	1.97 (0.77–5.29)	0.15
CC	25 (38.5)	16 (24.6)	3.05 (1.11–8.84)	0.03*
AC + CC	56 (86.2)	47 (72.3)	2.35 (0.97–6.0)	0.05*
A Alel	49 (37.7)	67 (51.5)	Reference	
C Alel	81 (62.3)	63 (48.5)	1.75 (1.07–2.88)	0.02*
rs1570360 (− 1154G > A)				
GG	3 (4.6)	10 (15.4)	Reference	
GA	25 (38.5)	30 (46.2)	2.13 (0.53–11.20)	0.26
AA	37 (56.9)	25 (38.5)	3.49 (0.89–18.10)	0.06*
GA + AA	62 (95.4)	57 (87.7)	2.07 (0.56–10.32)	0.26
G Alel	31 (23.8)	50 (38.4)	Reference	
A Alel	99 (76.1)	80 (61.5)	1.74 (1.01–3.01)	0.04*
rs2010963 (+ 405G > C)				
CC	9 (13.8)	9 (13.8)	Reference	
CG	30 (46.2)	33 (50.8)	0.91 (0.31–2.66)	0.85
GG	26 (40.0)	23 (35.4)	1.12 (0.37–3.41)	0.82
CG + GG	56 (86.2)	56 (86.2)	0.99 (0.33–2.78)	1.00
C Alel	48 (36.9)	51 (39.2)	Reference	
G Alel	82 (63.1)	79 (60.8)	1.10 (0.66–1.82)	0.70
rs3025039 (+ 936C > T)				
CC	49 (75.4)	43 (66.2)	Reference	
CT	15 (23.1)	20 (30.8)	0.66 (0.29–1.45)	0.29
TT	1 (1.5)	2 (3.1)	0.46 (0.01–5.98)	0.49
CT + TT	16 (24.6)	22 (33.9)	0.64 (0.29–1.37)	0.24
C Alel	113 (86.9)	106 (81.5)	Reference	
T Alel	17 (13.1)	24 (18.5)	0.66 (0.33–1.30)	0.23
rs25648 (c.534C > T)				
CC	49 (75.4)	51 (78.5)	Reference	
CT	16 (24.6)	12 (18.5)	0.72 (0.31–1.68)	0.18
TT	0	2 (3.1)	–	–
CT + TT	16 (24.6)	14 (21.6)	0.84 (0.37–1.90)	0.68
C Alel	114 (87.7)	114 (87.7)	Reference	
T Alel	16 (12.3)	16 (12.3)	1 (0.47–2.12)	1.00

OR odds ratio, CI confidence interval

**p* values ≤ 0.05 were considered statistically significant

Discussion

One of the important underlying mechanisms of HMB is the disruption of endometrial angiogenesis, and the main regulatory factor controlling angiogenesis is VEGF. In light of the studies suggesting an association between VEGF polymorphisms and diseases in which angiogenesis is of critical importance [12, 13, 17–19] this study aimed to investigate the significance of SNPs in the VEGF (rs699947 (− 2578C > A), rs1570360 (− 1154G > A) rs2010963 (+ 405G > C), rs3025039 (+ 936C > T), rs25648 (c.534C > T)

in HMB. Our results indicated an increased risk for HMB in patients with CC, AC + CC genotype, and C allele of the − 2578C > A polymorphism and AA genotype and A allele of the − 1154G > A polymorphism (*p* < 0.05).

In 2005, Mints et al. [1] conducted a prospective clinical study, which included 24 IM patients and 18 healthy fertile women, to analyze the expression of VEGF-A and its receptors (VEGFR-1 and VEGFR-2) in the endometrial blood vessels of patients with idiopathic menorrhagia (IM) in endometrial biopsy materials. It was found that the vascular expression of VEGF-A, VEGFR-1, and VEGFR-2

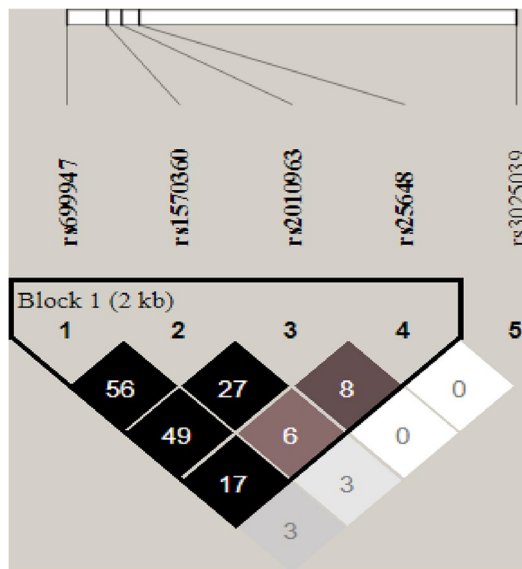


Fig. 1 Linkage disequilibrium plot created by Haploview 4.2

in capillaries was 1.8-fold, 1.8-fold, and 2.0-fold higher, respectively, in the IM group. It was also shown that VEGF-A and CD34 co-localized. Based on this finding, the authors emphasized that VEGF-A is expressed only in endothelial cells, not in pericytes or other cells adjacent to capillaries. Another finding was that the number of VEGFR-2-positive arterioles was twice as high in the IM group. As a result, they stated that up-regulation of VEGF-A and VEGFR-1 and VEGFR-2 in capillaries in menorrhagia may play a role in abnormal endometrial vascular structure and permeability.

In 2007, Mints et al. [10] planned a study, which included 24 women with IM and 18 healthy women, to investigate whether the growth, structure, or arrangement of endometrial blood vessels is abnormal in women with IM. Immunohistochemical staining was performed on endometrial biopsy samples collected from the participants, and the levels of CD34, CD31, von Willebrand factor, VEGF-A, and VEGFR-1 and VEGFR-2 were determined. In patients and controls, endothelial staining for CD34, CD31, and von Willebrand factor revealed focal gaps in endometrial vessels. Electron and confocal microscopy results demonstrated that

perivascular cells, possibly pericytes, were covering these gaps in the vessel wall. It was found that the relative size of the gaps was significantly greater and vessel circumference was larger in patients with IM than in controls, and more vessels were positive for VEGF-A and VEGFR-1 and VEGFR-2 in patients than in controls. Gap size was significantly correlated with the number of vessels expressing VEGF-A or VEGFR-1. Based on these observations, the authors suggested that IM may be associated with dysregulation of the VEGF signaling pathway, and that such dysregulation may be a mechanism for the formation of gaps and may contribute to HMB by rendering microvessels fragile.

In a 2010 study by Mints et al. [11], it was found that angiopoietin-1 level was upregulated in the secretory phase of the menstrual cycle in patients with HMB. The authors found a positive correlation between densities of angiopoietin-1 positive vessels and VEGFR-3 but did not detect a correlation between gap sizes and angiopoietin. Based on these findings, they reemphasized that angiopoietins play a minor role in the presence and size of the gap and that the VEGF signaling pathway is the most important factor in this regard.

Anderson et al. [5] conducted a prospective study and investigated whether the endometrial microvessels were fragile due to low pericyte coverage in patients with HMB-E and investigated whether this situation was associated with VEGF-A expression in microvessels. A total of ten women with normal menstrual cycles and a history of HMB-E of less than 5 years and 17 healthy women with normal menstrual cycles were included in the study. Those with a PBAC score of > 100 were included in the HMB group, and those with a PBAC score of < 80 were included in the control group. After the last menstrual period, the hormone analysis, and histological evaluation of endometrial biopsies, it was determined that five patients from the HMB group, eight patients from the control group were in the proliferative phase, and nine patients and five people from the control group were in the secretory phase of their menstrual cycle. Immunohistochemical smooth muscle actin- α (SMA α) staining was used to evaluate the extent of pericyte coverage in vessels, and image analysis (microvascular density) of endometrial biopsies was performed. Although the groups did

Table 4 Haplotype distributions of VEGF gene polymorphisms rs699947, rs1570360, rs2010963, and rs25648

Haplotype	Frequency (%)	HMB group, n=65 (%)	Control group, n=65 (%)	P	OR (95% CI)
CAAC	38.1	48 (36.9)	51 (39.2)	0.737	0.918 (0.555–1.517)
AGGC	31.2	31 (23.8)	50 (38.5)	0.012*	0.505 (0.295–0.864)
CAGC	17.3	33 (25.4)	12 (9.2)	0.0005*	3.387 (1.659–6.917)
AAGT	12.3	16 (12.3)	16 (12.3)	0.981	1.009 (0.481–2.116)

OR odds ratio, CI confidence interval

*p values ≤ 0.05 were considered statistically significant

not differ in terms of microvascular density, the number of SMA α -positive microvessels during the proliferative phase was significantly lower in patients with HMB-E than in the control group ($P=0.005$). A significant negative correlation was observed between the number of VEGF-A-positive microvessels and the number of microvessels with pericyte coverage ($r=0.8$; $P=0.04$). In addition, the endothelial cell layer in HMB-E patients was found to be significantly thicker than in the control group. Therefore, the authors reported that upregulation of VEGF-A in HMB-E is associated with low pericyte coverage, which may cause vessel fragility during the intense angiogenesis period in the proliferative phase, which may possibly lead to excessive blood loss.

Polymorphisms in regulatory regions of genes can alter gene expression through effects on transcription or RNA stability. It has been shown that there is a relationship between these functionally important polymorphisms and VEGF protein production. In addition to the literature on the role of VEGF polymorphisms and angiogenesis, studies on the specific VEGF polymorphisms have shown that VEGF-2578A > C (at the promoter region), - 1154G > A (at the promoter region), + 405G > C (at 5'UTR) and 936C > T (at 3'UTR) alter VEGF expression [13], and the studies by Dong et al. and Vural et al. demonstrated that the - 2578C, - 1154G and + 405G alleles are associated with increased VEGF protein levels [20, 21], corroborating our findings regarding the upregulation of VEGF-A in HMB-E, which is associated with vessel fragility and consequent excessive blood loss.

Despite the numerous studies in the literature on the relationship of VEGF and its receptors with HMB, there is no study in the literature on the role of *VEGF* polymorphism in HMB, and ours is the first one to investigate the effect of *VEGF* polymorphisms in HMB. *VEGF* polymorphisms were previously studied in cardiovascular diseases, essential hypertension, certain cancer types, psoriasis, and endometriosis. In addition, the + 405G > C allele was proposed as a candidate SNP associated with the pathogenesis of various female reproductive system diseases such as preeclampsia, endometriosis, recurrent pregnancy loss, and ovarian hyperstimulation syndrome following controlled ovarian stimulation [13]. Although being a unique study represents the power of our work, we did not have the opportunity to discuss or compare our results with similar articles.

The most important limitation of the current study is the small sample size, which can also be held responsible for the controversial results for different pathologies in other studies in the literature. Besides, the fact that these studies were performed on distinct ethnic groups and different diseases, and identified various haplotypes may have led to conflicting results. These diverse results suggest that disease tendency may stem from not a single polymorphism alone, but a combined effect of polymorphisms and that haplotype effect may

play a more significant role in disease tendency than single polymorphisms. In our study, during our analysis of the - 2578C > A, - 1154G > A, + 405G > C and 534C > T polymorphisms in the *VEGF*, we identified four haplotypes: CACC (38.1%), AGGC (31.2%), CAGC (17.3%), AAGT (12.3%). AGGC haplotype was less prevalent in HMB patients (23.8% vs 38.5%) while the CAGC haplotype was more prevalent in the HMB group (25.4% vs 9.2%).

Our study on VEGF polymorphisms in Turkish women with HMB demonstrated that there is an increased risk associated with the C allele, CC genotype, and AC + CC genotypes of - 2578A > C polymorphism, and the A allele, AA genotype, and CAGC haplotype of - 1154G > A polymorphism, whereas, the AGGC haplotype has a protective role. However, further studies with a larger sample size are required to demonstrate a more definitive association.

Author contribution İK: project development, data collection, data analysis, manuscript writing, editing. NSG: project development, reduction, editing.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Ankara Yıldırım Betazıt University Human Ethical Committee.

Informed consent Signed informed consent was obtained from all patients before starting study.

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